

Characteristics of normal cell division

- Anchorage dependence
 - Cells must be attached to a solid surface to divide
- Density dependence
 - Cells stop dividing when too dense
- Growth factors
 - Signals that regulate cell cycle
- Senescence
 - After a finite # divisions, cell self-destruct (**apoptosis**)
 - Irreparable DNA or membrane damage
 - Shortened telomeres

Primary culture of normal cells

- **Growth factor dependence**

EXPERIMENT

- 1 A sample of connective tissue was cut up into small pieces.
- 2 Enzymes were used to digest the extracellular matrix, resulting in a suspension of free fibroblast cells.
- 3 Cells were transferred to sterile culture vessels containing a basic growth medium consisting of glucose, amino acids, salts, and antibiotics (as a precaution against bacterial growth). PDGF (platelet-derived growth factor) was added to half of the vessels (T-flasks). The culture vessels were incubated at 37°C.
- 4 Cells cultured without PDGF did nothing. Cells cultured **with** PDGF attached to the vessel, flattened out, and began dividing. [**Growth-factor dependent growth**]

Scalpel
Petri plate

Figure 12.17

Primary culture of normal cells

- **Anchorage dependence; Density-dependent inhibition; & Senescence**

- 4 Cells cultured **with** PDGF attached to the vessel, flattened out, and began dividing. [**Growth-factor dependent growth**]

Cells anchor to dish surface and divide. [**anchorage dependence**]

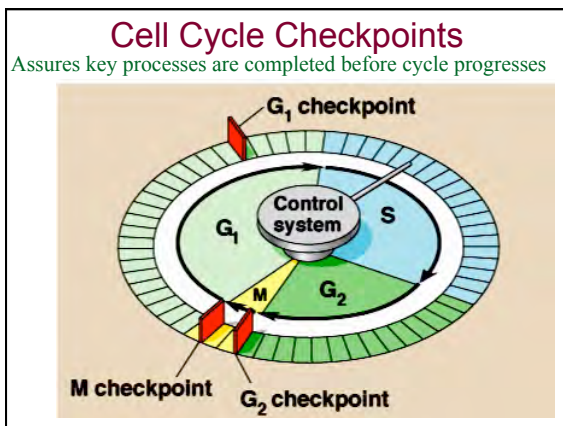
When cells have formed a complete single layer, they stop dividing. [**Density-dependent inhibition**]

25 µm

If some cells are scraped away, the remaining cells divide to fill the gap and then stop. [**Density-dependent inhibition**]

- 5 But even with low cell density, available surface, and added growth factors, after 20–50 cycles, the cells stop dividing, ball up, and detach. [**Senescence**]

Figure 12.18 A



Cell Cycle Checkpoints

Assures key processes are completed before cycle progresses

- **G₁ checkpoint:**
 - ✓ Sufficient growth & reserves to support replication
 - ✓ Pre-replication check for DNA damage
 - ✓ Internal clock
 - ✓ External growth factors and/or inhibitors
- **G₂ checkpoint:**
 - ✓ Sufficient growth & reserves to support mitosis & cytokinesis
 - ✓ Duplication of centrosomes
 - ✓ Replication of DNA
 - ✓ Pre-mitotic check for DNA damage
- **M checkpoint**
 - ✓ Spindle formed & functioning
 - ✓ Chromosome kinetochores correctly attached to spindle
 - ✓ Chromosomes properly aligned & untangled on metaphase plate

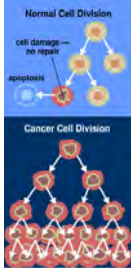
Transformation: damage to checkpoint mechanisms cause abnormal cell division

“Hallmarks of Cancer”:

- Growth independent of external growth regulators
 - Loss of anchorage & density dependence
 - Uncoordinated with surrounding tissues or the body
- Growth without stopping at checkpoints
- Avoidance of apoptosis despite cell/DNA damage
- Unlimited number of cell divisions
 - Activation of telomerase

Other indicators:

- De-differentiation
- Δ cytoskeleton → Δ morphology & motility
- Angiogenesis — induced growth of blood vessels to support increased metabolic demands of hyper-growth



Primary culture of *transformed* cells

- Cancer cells exhibit neither density-dependent inhibition nor anchorage dependence
- And have only limited dependence on growth factors

Cancer cells usually continue to divide well beyond a single layer, forming a clump of overlapping cells.

Many transformed cell lines can also be cultured as liquid cell suspensions with no need for attachment substrate.


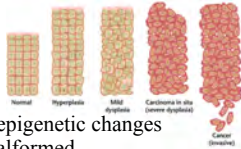


Figure 12.18 B

- **Transformed cells are also immortalized — showing no senescence**
 - E.g., the HeLa cell line was cultured from a tumor removed from Henrietta Lacks back in 1951. It is still growing in labs all over the world.

Stages of tumor progression



1. **Hyperplasia:** over-production of normal-looking cells.
2. **Dysplasia:** additional genetic/epigenetic changes lead to abnormal growth of malformed, disorganized cells.
3. **Solid tumor *in situ*:** cells are even more malformed and de-differentiated. Growth extends from original mass into the tissue.
4. **Malignancy (cancer):** cells detach and penetrate basal lamina into other tissues. May enter lymphatic or circulatory system and reach other organs to start new tumors.

Stages of tumor progression

- Metastasis: spread of malignant cells from original tissue

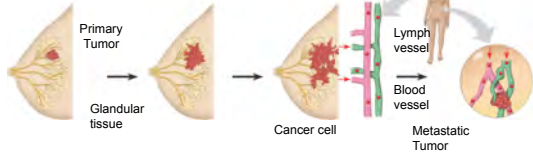
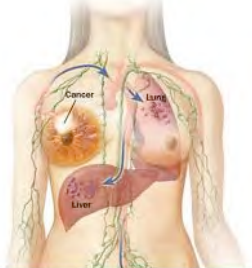


Figure 12.19

Stages of tumor progression

- Metastasis: spread of malignant cells from original tissue




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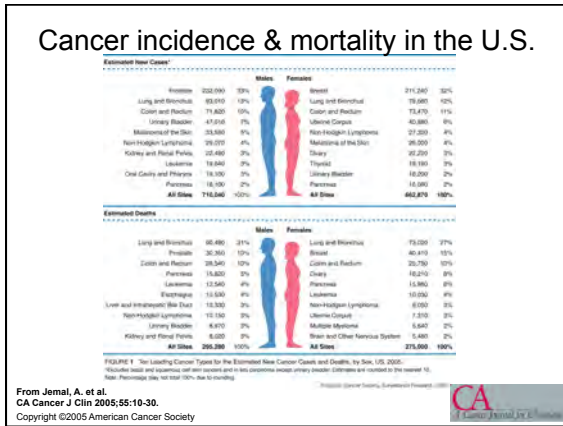
Types of tumors

Classification based upon tissue of origin

- “Solid tumors”
 - **Carcinoma:** epithelial cells
 - 80–90% of all cancers
 - **Sarcoma:** muscle or connective tissue
- Others
 - **Leukemia/Lymphoma/Myeloma:** bone marrow
 - **Glioma:** brain
 - **Choriocarcinoma:** placenta



Pulmonary carcinoma *in situ*



Transformation requires a series of non-lethal mutations within a specific cell line

- Turn on “on-switches”
 - Dominant mutations: proto-oncogenes ⇒ **oncogenes**
 - Bypass checkpoints
- Turn off “off-switches”
 - Recessive mutations: inactivate **tumor suppressors**
 - Remove checkpoints
- All cancers involve mutations in one or more oncogene and one or more tumor suppressors.

EFFECTS OF MUTATIONS

Protein overexpressed → Cell cycle overstimulated → Increased cell division

Protein absent → Cell cycle not inhibited

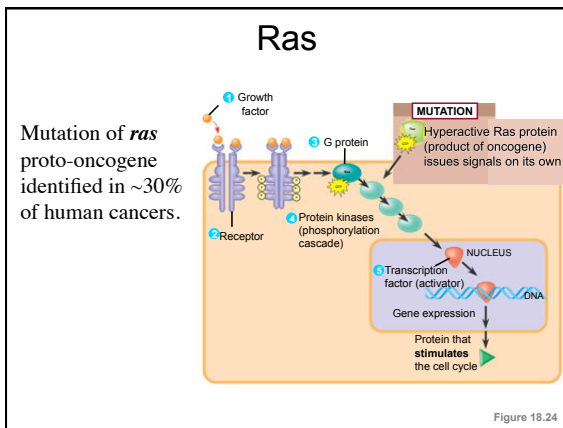
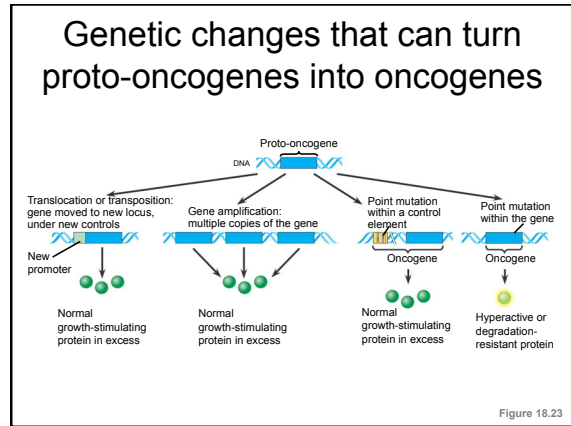
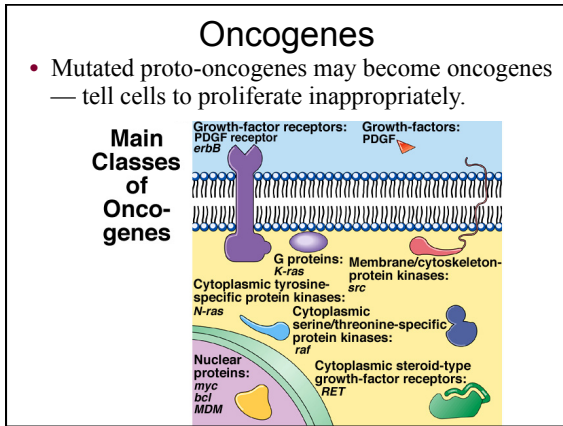
- ### Sources of mutations
- Spontaneous
 - Induced — mutagens/carcinogens
 - Radiation
 - UV — mostly point mutations
 - X-rays — translocations
 - Endogenous chemicals
 - Reactive oxygen species (ROS) → alter DNA bases
 - Chronic inflammation
 - Fat metabolism
 - Exogenous chemicals
 - Bind to DNA → replication & transcription errors
 - Benzo-pyrene from tobacco smoke
 - Aflatoxins from food-borne fungi
 - Viruses
 - Inserted pro-viruses
 - Viral-induced growth factors
 - Genetic carry-over from prior host cells

World Health Organization
Cancer Fact Sheet
<http://www.who.int/mediacentre/factsheets/fs297/en/>
Fact sheet N°297, Updated February 2015

- ❑ **Cancers figure among the leading causes of morbidity and mortality** worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012.
 - The number of new cases is expected to **rise** by about 70% over the next 2 decades. [to 22 million]
- ❑ Around **one third** of cancer deaths are due to the 5 leading **behavioral and dietary risks**: tobacco use, obesity, low fruit and vegetable intake, lack of physical activity, alcohol use.
- ❑ **Tobacco use is the most important risk factor** for cancer causing around 20% of global cancer deaths and around 70% of global lung cancer deaths.
 - Tobacco-related cancers, combined with tobacco-related diseases including cardiovascular and chronic lung diseases, make **tobacco use the leading cause of preventable deaths in the world.** [Even without effects of 2nd- & 3rd-hand smoking.]

- ### Oncogenes
- **Proto-oncogene**: normal gene functions in stimulating cell growth or viability, esp. for embryogenesis & organogenesis.
 - Mutated form = **oncogene**: stimulates unregulated cell division or immortalization.
 - **Presence** of the oncogene or oncogene product ⇒ ↑ probability of transformation

- ### Oncogenes
- **Presence** of the oncogene or oncogene product ⇒ ↑ probability of transformation
 - **Types of oncogenes**:
 - Growth factors
 - Growth factor receptor (**HER2**)
 - G-proteins (**Ras**)
 - Receptor-associated kinases (**Src**)
 - Transcription factors (**Myc**)
 - Telomerase activators
 - Apoptosis-regulating proteins (**Bcl-2**)

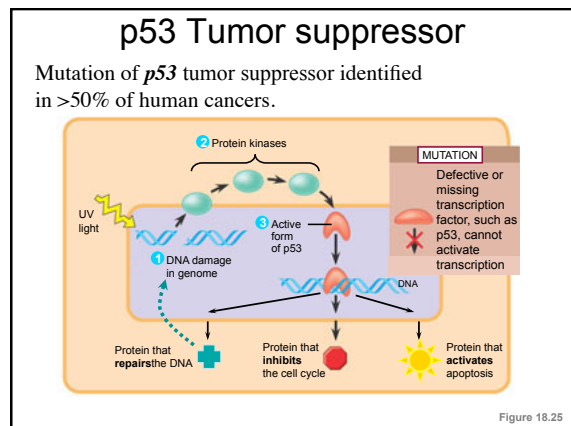


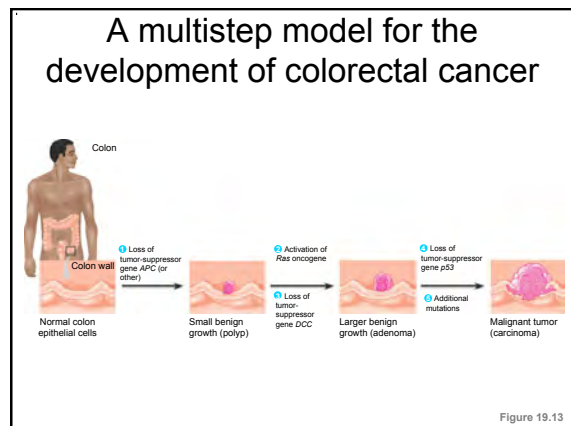
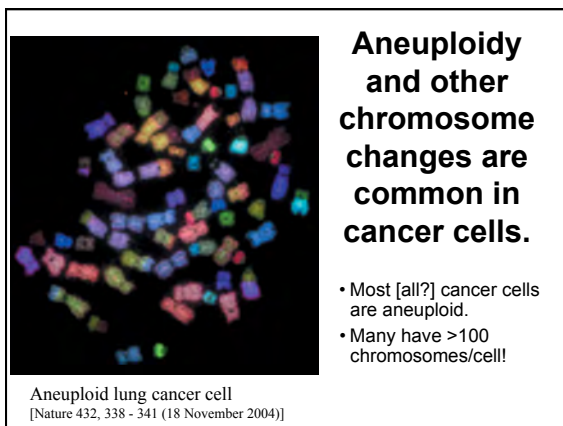
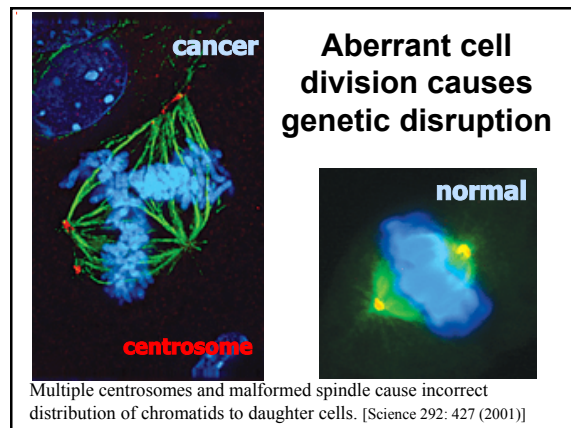
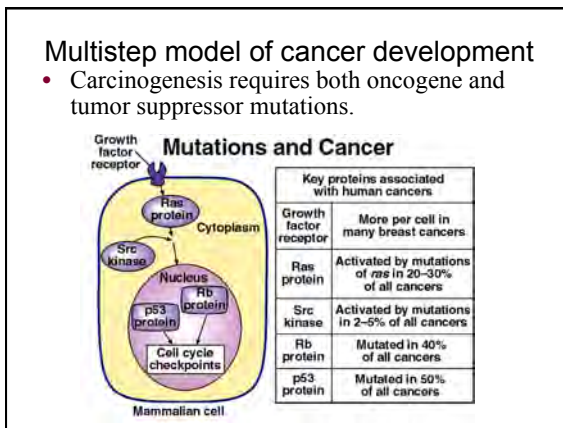
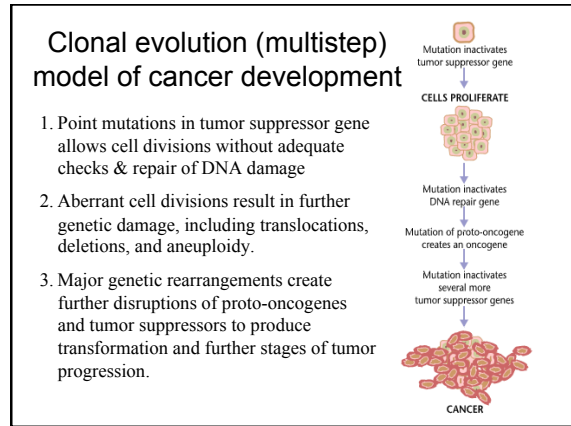
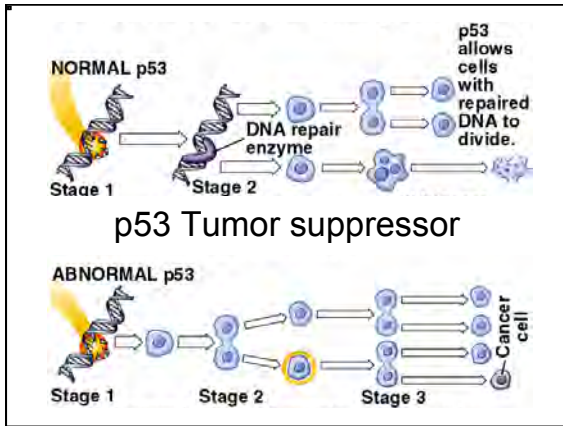
Tumor suppressors

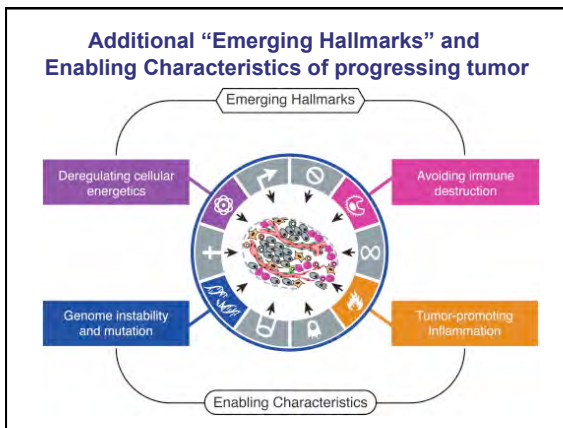
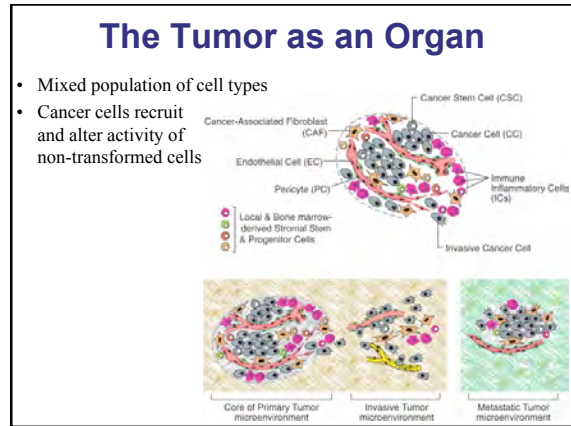
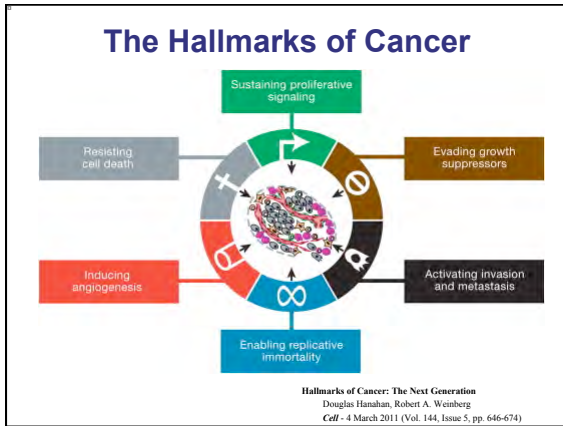
- **Tumor suppressors:** normal gene functions in delaying cell growth, locating or repairing damaged DNA, or initiating apoptosis of irreparably damaged or senescent cells.
- Mutated form is inactive: unable to regulate cycle, detect or repair genetic damage, or divert to self-destruct.
- Absence of the tumor suppressor \Rightarrow \uparrow probability of transformation

Tumor suppressors

- Absence of the tumor suppressor \Rightarrow \uparrow probability of transformation
- **Types of tumor suppressors:**
 - Transcription factors (*p53*)
 - Factors that restrict access of transcription factors (*APC*)
 - Factors that block effects of transcription factors (*Rb*)
 - DNA repair (*BRCA*)







What is cancer?

- Unrestricted proliferation of a cell line
 - Displacement of healthy tissues
 - Pressure on confined tissues
 - Over-consumption of resources
- Metastasis
 - Spreading
- Disrupted gene expression
 - De-differentiation
 - Loss of normal function
 - Inappropriate production of bioactive substances

